A Spectrophotometric Study of Actomyosin

By Jane S. Robb, M.D., Sc.D., Marlin Grubb, B.S. and Helena Braunfelds

It is known that when actomyosin and adenosine triphosphate (ATP) interact, the ultraviolet absorption is no longer that characteristic either of actomyosin or of ATP. This report deals with a spectrophotometric study of interactions of various chemicals and/or drugs with actomyosin. The intention was to discover whether these agents alter the absorption, i.e., optical density (O.D.) of actomyosin solutions and if so, whether all the agents produce similar changes.

As previously reported, we have observed that shortening of compressed actomyosin threads could be triggered by a variety of substances in the absence of adenosine triphosphate (ATP). Spectrophotometric examinations of diluted actomyosin were made, for it seemed possible that the absorption spectra of this substance might be altered, and in different ways, by the various agents.

METHODS

Standard procedures for spectrophotometric examinations as outlined by the Beckman Laboratory were followed except that we did not work in a room having constant temperature and humidity. However, we have accumulated reproducible data in various seasons, under various weather conditions. A stock solution of actomyosin, prepared according to Szent-Györgyi's method described by Hayashi, is made up to 0.6 M concentration by addition of crystalline KCl and to this an equal amount of glycerine is added. Total solids (corrected for salt content and without glycerine present) for our skeletal actomyosin extracts average 1.0 per cent. For spectrophotometric work the comparison cuvette is filled with a solution containing exactly the amounts of water, KCl, and glycerine which are present in the actomyosin solvent. The various drugs studied were also added to the comparison cuvette. The stock actomyosin is diluted with 0.6 M KCl to make a 0.1 per cent solution on the basis of total solids. The resulting pH is 7.5. Centrifugation of 20,000 g, (or filtering through Whatman No. 1 paper) of these diluted preparations does not alter the degree of absorption.

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RESULTS

The Beer-Lambert Law, expressed by a straight line relationship between optical density (O.D.) and percentage concentration, is found to hold for all agents used in this study.

Figure 1 shows the absorption curves for skeletal actomyosin at pH 7.5 and pH 12. At pH 7.5, apart from end absorption, there is a maximum at m/2 276. When the pH is 12 a shoulder extends from m/2 242 to 250 and also there are low maxima at 284 and 292 m/. These curves are similar to those for crystalline beef albumin published by Sizer and Peacock whose demonstration of the relation of crystalline beef albumin absorption to that of tyrosin and the shift of maximum density at pH 12 for both substances is well known.

The absorption for ATP, .002 per cent, is shown (fig. 2A) and for ATP, .005 per cent, (fig. 2B) the X maximum is at 260 m/ (curves marked ATP). The X maximum (276 m/) for 0.1 per cent actomyosin is shown in the same figures (curves marked SK. AM.). Observed absorption for combinations of actomyosin and ATP within the same cuvette are marked SK. AM. + ATP. If one adds the absorption for the ATP to that for the skeletal actomyosin the calculated absorption for the two in combination would fall along the unconnected plus signs. The dotted curve marked “15 minutes” shows the values actually read, readings being finished 15 minutes after mixing of the actomyosin and ATP. The “15 minute” curves are similar to but not identical with the calculated positions. Always the 15 minute curve maximum was somewhat lower than the calculated maximum. The beaker containing the mixture of ATP and actomyosin was placed in a refrig-
SPECTROPHOTOMETRIC STUDY OF ACTOMYOSIN

Fig. 1. Absorption spectra of 0.1 per cent actomyosin at pH 7.5 and 12.0.

Fig. 2. Curves showing absorption of ATP, skeletal actomyosin, Sk. AM, and their mixture. ATP concentration in A is 0.002 and in B is 0.005 per cent. Discussion in text.
weighed crystals of sodium chloride and to the third weighed crystals of calcium chloride were added. Thus, when the effects of NaCl and CaCl₂ on actomyosin were tested, one compared to actomyosin controls in the very same cuvette as well as to a standard control in a matched cuvette. Initial readings from the various cuvettes were practically identical. In figure 3 the curve connecting open circles indicates the absorption of the actomyosin. Two hours later the values are so alike that two separate curves were not drawn. The lowest curve shows that addition of NaCl always depressed the curve, i.e., allowed more light to pass. This effect was immediate and did not progress with time. The upper connected curve shows that absorption became greater when calcium chloride was added. This change was progressive (see upper curve and small bars above it). The addition of small amounts of these salts did not cause observable precipitation although very large amounts did. The point of maximum absorption, μ₂76, and the pH are not changed by the addition of these salts.

Two types of response occur when actomyosin combines with agents which induce shortening of compressed threads. In figure 4, solid dots indicate the control absorption curve for actomyosin. The lower curve presents the absorption curve for digitoxin. When the data represented in these two curves are added, the upper “calculated” curve results. The curve produced by connecting open circles is the absorption actually read. Thus the effect is to reduce absorption without shifting the maximum. The effects judged by appearance of the spectral curves are similar for sodium chloride and digitoxin. Other substances producing this type of change are alcohol, histamine, epinephrine, salyrgan, thyroxin, thyronin and vitamin B₁₂.

The situation is different in the case of veratridine. In figure 5 the line connecting solid circles indicates the absorption of actomyosin alone. The lower curve indicates absorption of veratridine. The upper curve is calculated by summing the absorption values for veratridine and actomyosin. The maximum for this calculated curve is to the right of the maximum for veratridine and to the left of the maximum for

![Image](http://circres.ahajournals.org/)

**Fig. 3.** Absorption spectra of actomyosin alone and in mixtures with NaCl and CaCl₂. Details in text.

**Fig. 4.** Absorption spectra of digitoxin (Crystogidigin-Lilly) 0.1 per cent actomyosin, and their combination. Details in text.
SPECTROPHOTOMETRIC STUDY OF ACTOMYOSIN

actomyosin. The curve connecting open circles gives actual readings when actomyosin and veratridine were present in the same cuvette. It is unlike the upper, calculated curve, in three respects: (a) it consistently lies below the calculated curve, (b) the maximum has shifted from 272 to 264, and (c) it falls off more steeply (to the right) from the maximum. Other agents which lessen the absorption and shift the maximum are allostrophanthidine, acetyl \( \beta \) methyl choline, cortone acetate, ouabain and Paveril.

DISCUSSION

Ravikovich and associates, and Tarver and Morales have also reported the ultraviolet absorption of actomyosin solutions and have found maxima at 277.5 \( \text{m} \mu \) and "near 280" \( \text{m} \mu \) respectively. The latter authors also describe a shoulder "near 230" \( \text{m} \mu \).

It is commonly supposed that absorption in the range of 250 to 300 \( \text{m} \mu \) is due to one of three aromatic amino acids, phenylalanine (258 \( \text{m} \mu \)), tryptophane (275 \( \text{m} \mu \)) or tyrosine (274 \( \text{m} \mu \)). Chemical analyses reported by Weber and Portzehl indicate that seventeen amino acids can be identified in myosin solutions. These are tyrosine, tryptophane, phenylalanine, cystin, methionin, glycine, alanine, valine, leucines, proline, serine, threonine, histidine, arginine, lysine, glutamic acid and aspartic acid. In addition, we find diiodotyrosine in hydrolyzed heart actomyosin.

Sizer and Peacock found that there was negligible shift of absorption maxima with shift of pH in the case of phenylalanine and tryptophane. Because actomyosin absorption does shift as pH becomes alkaline, we believe tyrosine has a considerable influence. The broadness of the maximum at pH 7.5 and the low peak at 284 when pH is 12, lead us to believe tryptophane is also a factor.

More recent studies, discussed by Neurath and Bailey indicate that there is also absorption in the U.V. not due to the aromatic amino acids. There is no agreement concerning the explanation of this additional absorption. Absorption ratios, 250/260 and 280/260 are established for certain products. The 280/260 ratio for proteins is said to be about 1.7. We have read absorbances for crystalline beef albumin (Armour) at pH 7.5 and find the 250/260 and 280/260 ratios to be, 0.82 and 1.86 respectively. For commercially prepared L-tyrosine (pH 7.5) these ratios are 0.44 and 1.89; for tryptophane (pH 7.5) they are 0.43 and 1.75 and for phenylalanine (pH 7.5) they are 0.94 and 0.90 respectively. When these three amino acids are all present the absorptions (assuming no interaction) will be additive. According to Weber and Portzehl the aromatic amino acid content in rabbit skeletal myosin (results calculated on N content of 16.7 per cent, reported as residues per 100 Gm.) is, tyrosine .0188, tryptophane .0039 and phenylalanine .0262. Commercial preparations of these amino acids were combined in these proportions, put into solution and read at various pH's, and the ratios calculated. Table 1 presents the results and also gives similar data for actomyosin.

Weber and Portzehl's figures for amino acid content are for myosin, and the amino acid content of actomyosin probably is different. However, this study together with observations discussed by Neurath and Bailey leave the impression that the entire absorption of actomyosin between \( \text{m} \mu \) 250 and 300 is not due to the aromatic amino acids.

Tarver and Morales studied the reaction of actomyosin in the presence of Ca\(^{++}\) (0.001 M)
Table 1.—Optical Density Ratios

<table>
<thead>
<tr>
<th>Amino acid mixture of Phenylic, Tyrosinc, Tryptophanc</th>
<th>pH</th>
<th>Actomyosin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.5</td>
<td>7.5</td>
</tr>
<tr>
<td>250/260</td>
<td>0.54</td>
<td>0.56</td>
</tr>
<tr>
<td>280/260</td>
<td>1.48</td>
<td>1.50</td>
</tr>
</tbody>
</table>

discovers a rapid reversible process. This is thought "not to be due to a change in the amount of light scattered, but as a true change in the absorption spectrum (i.e., there is a different direction of change in optical density at different wave lengths, positive for wave lengths less than 255 mµ and negative for wave lengths greater). This, however, we regard as a very tentative result." Tarver and Morales do not identify this phenomenon with one described by Ravikovich who used greater concentrations, observed a maximum deflection in an hour and incomplete recovery in 20 hours. We quote these authors to establish that others note changes in actomyosin induced by Ca++

Obviously the change we observe, increasing up to 24 hours, is not that reported by Tarver and Morales, nor do our readings indicate that the change is reversible.

If the change in absorption of actomyosin following addition of NaCl were due to dilution and to that exclusively, the 250/260 and 280/260 ratios should be unaltered. Table 2 presents the data for one experiment where NaCl and also CaCl₂ additions were observed immediately and after 3 and 5½ hour intervals. It is evident that the ratios for actomyosin absorption are constant while those for combination with NaCl and with CaCl₂ are altered. When either NaCl or CaCl₂ are added to saturation precipitation occurs. Following filtration (through porcelain plus two layers of Whatman no. 1) the absorption of the cleared solutions was read. The 250/260 following NaCl addition to saturation then becomes 1.45 and following CaCl₂ addition becomes 1.30 while the 280/260 ratios are 0.42 and 0.48 respectively. Hence, the entire effect of CaCl₂ may be due to gradually increasing precipitation of a material which has been masking the absorption of the substance which CaCl₂ does not precipitate. It is not immediately evident why the early change due to NaCl (slight decrease of 250/260 and a slight increase of 280/260) is in the opposite direction to that observed when saturation has removed a substance (presumably globulin) thus unmasking the absorption of another substance or of substances which are not removed by saturation with NaCl. When diluted actomyosin is filtered by suction through a combined paper and porcelain filter, and the resultant filtrate read, the ratios are 250/260, 1.39; 280/260, 0.47, indicating that a substance can be removed after which there is still absorption. When actomyosin is treated with perchloric acid, centrifuged, the supernatant neutralized with KOH and allowed to stand in the cold overnight, then recenterfuged and the supernatant read, the 250/260 ratio is 1.11 and the 280/260 ratio is 0.77. When the protein is removed after addition of ATP, the ratios were found to be 1.45 and 0.28 respectively. Our next procedure will be to determine whether these protein-free filtrates contain one or more than one substance. In any case, it appears that our thrice washed actomyosin is not composed entirely of protein.

Data for the experiment (fig. 2) in which actomyosin and ATP were combined is presented as table 3. Various investigators, Herrmann and Josepovits, Tarver and Morales, Deutsch and Nilsson, have stated that actomyosin dephosphorylates and deaminates adenosine triphosphate so that at equilibrium the spectrum (minus the protein absorption) is that of hypoxanthine derivatives. Many spectrophotometric studies of purines have been made by Kalckar who sets the λ maximum for hypoxanthine (acid) at 249/250 mµ. When we subtract the actomyosin absorption from the ATP plus actomyosin readings a smooth curve results. When the ATP concentration was
It is obvious that absorption is less in the pres-
were combined is due to change in 
Other substances producing curves when the absorption is lessened and the max-
We have calculated 250/260 and 280/260 
When possible, absorptions of various products 
actomyosin have been summated and the 
ihypotheses of the same chemicals with actomyosin.

When veratridine is combined with acto-
there is a difference in slope of the observed curve in the region of μ 277, compared either to the cal-

<table>
<thead>
<tr>
<th>Wave length μμ</th>
<th>250</th>
<th>260</th>
<th>280</th>
<th>250</th>
<th>260</th>
<th>280</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-1000 Actomyosin</td>
<td>0.158</td>
<td>0.167</td>
<td>0.201</td>
<td>0.01</td>
<td>1.20</td>
<td></td>
</tr>
<tr>
<td>1-20000 ATP</td>
<td>0.820</td>
<td>1.076</td>
<td>1.640</td>
<td>0.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-1000 AM 15 min.</td>
<td>0.987</td>
<td>1.248</td>
<td>0.365</td>
<td>0.79</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td>1-1000 AM 15 min.</td>
<td>1.188</td>
<td>1.410</td>
<td>0.744</td>
<td>0.84</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>1-20000 ATP observed</td>
<td>1.267</td>
<td>1.188</td>
<td>0.744</td>
<td>1.07</td>
<td>0.53</td>
<td></td>
</tr>
</tbody>
</table>

1-50,000, pH 7.5 the λ maximum at 15 minutes 
was at 257 μμ, the 250/260 ratio was 0.79, 
the 280/260 ratio was 0.17. When the ATP 
concentration was 1-20,000, pH 7.5, the λ maximum 
was 15 minutes was at 257 μμ, the 250/260 ratio 
was 0.78 and the 280/260 ratio was 0.15. If our 

<table>
<thead>
<tr>
<th>Substance plus actomyosin</th>
<th>Test substance plus actomyosin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actomyosin, skeletal, rabbit</td>
<td>0.95 1.20</td>
</tr>
<tr>
<td>Actomyosin, heart, beef</td>
<td>0.93 1.03</td>
</tr>
<tr>
<td>Alcohol, 0.5%</td>
<td>2.44 0.0027 0.95 0.95 0.21</td>
</tr>
<tr>
<td>Crystodigin, Lilly</td>
<td>2.34 0.07 1.11 1.09 1.12</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>0.90 1.85 0.93 1.320 0.94 0.40</td>
</tr>
<tr>
<td>Histamine</td>
<td>1.004 0.79 0.94 1.160 0.95 1.20</td>
</tr>
<tr>
<td>Salyrgan</td>
<td>0.91 1.19 0.91 1.190 0.92 1.20</td>
</tr>
<tr>
<td>Thyroxine</td>
<td>1.96 0.47 1.05 1.101 1.02 1.14</td>
</tr>
<tr>
<td>Thyonin*</td>
<td>2.18 0.65 1.021 1.15 1.04 1.17</td>
</tr>
<tr>
<td>Vitamin B1</td>
<td>0.83 0.77 0.91 1.020 0.90 1.07</td>
</tr>
<tr>
<td>Adenosine triphosphate</td>
<td>0.79 0.17 0.84 0.53 0.70 0.29</td>
</tr>
<tr>
<td>Adenosine 5 phosphorinic acid</td>
<td>0.80 0.15 1.10 0.50 0.84 0.56</td>
</tr>
<tr>
<td>Acetyl β methyl choline</td>
<td>0.90 0.0023 0.94 0.53 0.91 0.46</td>
</tr>
<tr>
<td>Allosteranthidin†</td>
<td>5.50 0.61 1.31 1.02 1.29 1.17</td>
</tr>
<tr>
<td>Cortone acetate</td>
<td>1.50 0.25 1.26 0.67 1.19 0.73</td>
</tr>
<tr>
<td>Ouabain</td>
<td>4.26 0.40 1.54 0.71 0.67 0.64</td>
</tr>
<tr>
<td>Paveril (Lilly)</td>
<td>2.60 0.34 1.23 0.96 1.10 0.88</td>
</tr>
<tr>
<td>Veratridine†</td>
<td>0.64 0.47 0.79 0.74 0.77 0.82</td>
</tr>
</tbody>
</table>

* Most kindly supplied by Smith, Kline & French Laboratories.
† Most kindly supplied by Dr. K. K. Chen, Lilly Research Laboratories.
‡ Most kindly supplied by Dr. Otto Krayer, Department of Pharmacology, Harvard University.
is that if two substances are present but not reacting the "calculated" and observed data would be similar but if reactions were occurring the calculated and actually read absorptions, and hence the ratios, would be dissimilar. These calculations are presented as table 4.

SUMMARY AND CONCLUSIONS

Using a Beckman DU Model Spectrophotometer, absorption spectra for actomyosin and for various amino acids, chemicals and/or drugs have been determined.

Data collected for ATP, tyrosine and actomyosin relating percentage concentration to absorption agree with the Beer-Lambert Law.

When the pH of skeletal actomyosin solution is 7.5 the maximum absorption is at 276 μm; when the pH is 12, different maxima are present; one a shoulder at 246 μm, the others at 284 and 292 μm.

When ATP (maximum absorption μm 260) is combined with actomyosin (maximum absorption μm 276) the immediate absorption is only slightly less than one would expect from simple addition of absorption data for the two substances. Later the divergence becomes definite, the observed absorption being far less than the calculated and the maximum shifting to about 257 μm.

Addition of crystalline sodium chloride to a given concentration of actomyosin depresses the absorption while similar addition of calcium chloride increases the absorption. This effect of calcium chloride is progressive for at least 24 hours.

Depression of absorption with no shift in maximum is one response when chemicals are combined with actomyosin. Some agents resulting in this change are sodium chloride, digitoxin, alcohol, histamine, epinephrine, vitamin B₁, thyroxine, thironin and Salyrgan.

A second type of response involves lessening of the degree of absorption and shift of the point of maximum absorption. Agents producing this effect are ATP, veratridine, ouabain, acetyl β methyl choline, cortone acetate, Paveril and alloproanthidin.

The only agent studied which increased absorption was calcium chloride.

The absorption ratios 250/260 and 280/260 of combinations of various substances with actomyosin are presented and discussed.

REFERENCES

A Spectrophotometric Study of Actomyosin
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